ALDEHYDE-PROTEIN ADDUCTS IN THE LIVER AS A RESULT OF ETHANOL-INDUCED OXIDATIVE STRESS

Onni Niemelä

Department of Clinical Chemistry, University of Oulu, FIN-90220 Oulu, and EP Central Hospital Laboratory, Seinäjoki, Finland

TABLE OF CONTENTS

1. ABSTRACT

A number of systems that generate oxygen free radicals and reactive aldehydic species are activated by excessive ethanol consumption. Recent studies from human alcoholics and from experimental animals have indicated that acetaldehyde and aldehydic products of lipid peroxidation, which are generated in such processes, can bind to proteins forming stable adducts. Adduct formation may lead to several adverse consequences, such as interference with protein function, stimulation of fibrogenesis, and induction of immune responses. The presence of protein adducts in the centrilobular region of the liver in alcohol abusers with an early phase of histological liver damage indicates that adduct formation is one of the key events in the pathogenesis of alcoholic liver disease. Dietary supplementation with fat and/or iron strikingly increases the amount of aldehyde-derived epitopes in the liver together with promotion of fibrogenesis. question what are the real adducts formed *in vivo* based on recent experiments carried out both in alcoholic patients and in experimental animals. The relationship between adduct formation and alcohol toxicity is also discussed.

2. INTRODUCTION

Evidence continues to grow indicating that reactive aldehydic products resulting from ethanol metabolism and ethanol-induced oxidative stress play a pivotal role in the pathogenesis of alcoholic liver injury (1-6). Reactive aldehydes and hydroxyl radicals, which may be generated during periods of heavy ethanol intake, are known for their ability to attack amino acid residues of proteins thereby forming both stable and unstable adducts with proteins and cellular constituents (1, 7-14). As a consequence, cellular functions may become disturbed together with damage to proteins, nucleic acids and lipids (9, 15-17). Several different types of adducts have been recently identified. The purpose of the present communication is to address the

3. FORMATION OF PROTEIN-ALDEHYDE ADDUCTS

3.1. Reactive compounds generated during ethanol metabolism

Adducts of proteins with *acetaldehyde*, the first metabolite of ethanol, have been described in a number of studies (table 1). Acetaldehyde forms adducts primarily via binding to reactive lysine residues of preferred target proteins (1,6,7,10,18). While the data on the reactivity of acetaldehyde at physiologically relevant concentrations have remained controversial, it appears that among acetaldehyde-exposed proteins those with abundant amounts of reactive lysine residues are readily modified even at low concentrations of acetaldehyde under appropriate reducing conditions (19,20). On the other hand, even in the absence of reducing agents stable cyclic imidazolidinone structures are generated as a result of a reaction between acetaldehyde and the free alpha-amino group of the aminoterminal valine of hemoglobin (21,22).

Aldehydic products of lipid peroxidation, such as *malondialdehyde (MDA)* and *4-hydroxynonenal (HNE)*, also form Schiff's base adducts with proteins (table 1). MDA is a highly reactive dialdehyde originating from nonenzymatic lipid peroxidation of a variety of unsaturated fatty acids, from lipid peroxidation that occurs during phagocytosis by monocytes and from arachidonic acid catabolism in thrombocytes (23,24). The free radical-

Table 1. Reactive compounds generating protein adducts in alcohol abusers.

Compound	Abbreviation	Reference
Acetaldehyde	AA	Nomura and Lieber 1981,[78] Medina et al. 1985,[79] Israel et al. 1986,[10] Behrens et al. 1988,[33] Niemelä et al. 1991,[65] 1994,[28] 1995,[76] Lin et al. 1988,[11] Halsted et al. 1993,[67] Holstege et al. 1994,[66] Yokoyama et al. 1995[61]
Malondialdehyde	MDA	French et al. 1993,[80] Niemelä et al. 1994,[28] 1995,[76] Tsukamoto et al. 1995[69]
4-hydroxynonenal	HNE	Kamimura et al. 1992,[81] French et al. 1993,[80] Niemelä et al. 1995,[76] Tsukamoto et al. 1995[69], Chen et al. 1998[82] Ohhira et al. 1998[83]
Malondialdehyde-acetaldehyde hybrid	MAA	Tuma et al. 1996[31] Xu et al. 1998[59]
Hydroxyethyl radical	HER	Moncada et al. 1994,[13] Clot et al. 1995,[32] 1996,[14] 1997 [84] Albano et al. 1996[85]

Table 2. Preferred target proteins for adduct formation in alcoholics.

Protein	Reference
Erythrocyte membrane proteins	Gaines et al. 1977[86]
Hemoglobin	Stevens et al. 1981,[7] San George and Hoberman 1986,[21] Niemelä and Israel 1992,[62] Peterson and Scott 1989[87]
Albumin	Donohue et al. 1983,[18] Israel et al. 1986[10]
Tubulin	McKinnon et al. 1987,[37] Jennett et al. 1989,[20] Smith et al. 1989,[38] Tuma et al. 1991[39]
Collagens	Jukkola and Niemelä 1989,[88] Behrens et al. 1989[89]
Lipoproteins	Haberland et al. 1988,[26] Steinberg et al. 1989,[27] Palinski et al. 1990,[23] Wehr et al. 1993,[58] Lin et al. 1995,[12] Paradis et al. 1996[90]
Ethanol-inducible cytochrome P450IIE1	Behrens et al. 1988,[33] Clot et al. 1996[14]
Ketosteroid reductase (37 kD)	Lin et al. 1988,[11] Zhu et al. 1996[36]

mediated oxidation of long-chain polyunsaturated fatty acids leads to the production of 4-hydroxynonenal, which can react with the sulfhydryl groups of proteins through a Michael addition type of mechanism (23-25). Oxidative modification of proteins with MDA and HNE have been demonstrated to occur *in vivo* on arterial vessel walls of atherosclerotic lesions (26,27). Similar epitopes have also been found from the liver specimens of patients with alcoholic liver disease (28) and from animals with experimental iron overload (29,30).

Tuma and coworkers have recently demonstrated the formation of *hybrid adducts with acetaldehyde and malondialdehyde*, designated as *MAA adducts*, in livers of ethanol fed rats (31). Such hybrid adducts may act in a synergistic manner and may also be involved in the mechanisms for stabilization of protein adducts *in vivo* (31). In addition, the appearance of *hydroxyethyl radicals*, a reactive species resulting from ethanol during its oxidation in the presence of iron, has also been described from liver microsomes of ethanol-fed animals (13,32).

3.2 Proteins involved in adduct formation

There seems to be several preferred target proteins for aldehyde attack *in vivo* (table 2). The continuously growing list of the primary targets include erythrocyte membrane proteins, hemoglobin, albumin, tubulin, lipoproteins, and collagens. Not surprisingly, adduct formation with acetaldehyde, hydroxyethyl radicals and ethanol-metabolizing cytochrome P450IIEI enzyme, seem to occur *in vivo* (14,33). Cytochrome enzymes are also known to be involved in the formation of the reactive compounds during ethanol metabolism (14,32-35). A 37 kD protein which has repeatedly been reported as a preferential binding site for acetaldehyde in Western blot experiments from liver fractions was recently identified as ketosteroid reductase, which catalyzes the reduction of key intermediates in bile acid biosynthesis (36).

4. CLINICAL SIGNIFICANCE OF PROTEIN MODIFICATIONS IN ALCOHOLICS

4.1. Functional consequences

Formation of protein adducts with reactive aldehydic products has provided a basis for new hypotheses to explain the pathogenesis of ALD (figure 1), which have been previously reviewed in detail by Tuma and Sorrell (6,9). Covalent binding to proteins is known to interfere with protein function particularly when there is a lysine residue in a functionally critical location, such as in tubulin and in lysine-dependent enzymes (9,20,37-41). Altered microtubule function may subsequently lead to an impairment in protein secretion and plasma membrane

507
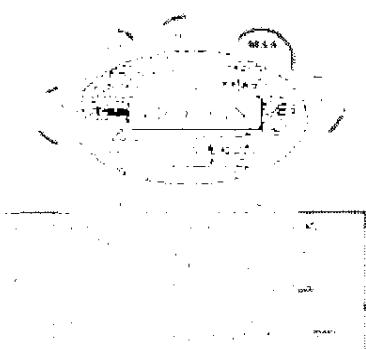
Figure 1. Schematic representation of various reactive components capable of attacking proteins to form adducts during ethanol metabolism. Protein binding with such compounds provides a basis to explain the pathogenesis of alcoholic liver disease and to diagnostic applications. See text for details. Abbreviations, AA, acetaldehyde, MDA, malondialdehyde, HNE, 4-hydroxynonenal, MAA, malondialdehyde-acetaldehyde hybrid adduct, HER, hydroxyethyl radical.assembly. Generation of reactive aldehydes may also contribute to ethanol-induced impairment in receptor-mediated endocytosis (42).

4.2 Stimulation of fibrogenesis

A number of cell culture studies have shown that aldehydic products derived from ethanol metabolism and lipid peroxidation can increase collagen mRNA levels and enhance the expression of connective tissue proteins (43-49). Acetaldehyde is able to increase the production of several extracellular matrix components (47,50). Reduction of adduct formation by scavengers of reducing equivalents has been shown to abolish such increases (44). Studies have further demonstrated that hepatic stellate cells (Ito cells) which are the primary source of extracellular matrix become readily activated under conditions involving enhanced oxidative stress and lipidperoxidation (49-52).

4.3. Stimulation of immune reactions

Aldehyde-protein adducts and hydroxyl radicals also stimulate immunological responses directed against the specific modifications of proteins (10,14,32,53-58). Studies have shown that chronic administration of ethanol to rats leads to the generation of circulating immunoglobulins with anti-acetaldehyde (10) adduct or anti-MAA-adduct (59) specificity. Similar immunoglobulins and autoantibodies recognizing cytochrome P450IIE1 hydroxyethyl radical adducts have also been found from the blood of human alcoholics (14,32). The highest titers of all such antibodies have been observed from patients with severe alcoholic liver disease (32,53-60). Characterization of the immunoglobulin isotypes involved in the above responses have revealed both IgA and IgG autoantibodies (32,56,60). In addition, immunization of experimental animals with aldehyde-protein conjugates has been shown to result in the production of antibodies recognizing protein-aldehyde condensates independently of the nature of the carrier protein (10,23). Such antibodies have considerable specificity towards aldehyde-lysine residues. Despite the small molecular weights of the aldehyde-amino acid condensates and the relatively small immunological differences between the various aldehyde-amino acid residues as haptens, antibodies with either anti-acetaldehyde or anti-malondialdehyde adduct specificity devoid of crossreactivity have been demonstrated in dot blot comparisons (28). It has further been shown that after immunization with acetaldehyde adducts, experimental liver disease can readily be induced by ethanol challenge to the immunized animals (61).

4.4. Protein adducts as biological markers

It is tempting to speculate that detection of ethanol-derived chemical condensation products could also serve as cumulative markers of ethanol intake (acetaldehyde adducts, hydroxyethyl radicals) or perhaps as markers of life style epitopes induced by enhanced oxidant stress) (figure 1). In some studies, antibody-based detection of protein-acetaldehyde adducts has been used as an approach to study the existence of such condensates in vivo. However, despite the fact that increased concentrations of acetaldehyde adducts have been found to occur in the erythrocytes of alcohol consumers, lack of sensitivity of the adduct measurements from blood proteins has so far hampered their clinical use as alcohol markers (62-64).

Methods have also been developed for the detection of aldehyde-derived condensation products from tissue specimens (65). Immunohistochemical stainings for acetaldehyde-protein adducts from the liver of human alcohol abusers have revealed positive staining, which is restricted to the centrilobular region in the early phase of liver disease (65). Samples from heavy drinkers with no obvious clinical, biochemical or light microscopical signs of alcoholic liver disease were found to contain such epitopes primarily in the cytosol of hepatocytes. With progression of ALD the staining becomes more widespread. Studies by Holstege et al have further shown the occurrence of sinusoidal acetaldehyde adducts in alcoholic patients (66). Sinusoidal acetaldehyde adducts were also related to a poor prognosis of such patients (66). Liver specimens from human alcoholics have also been found to contain protein adducts withaldehydic products of lipid peroxidation (28). In comparisons relying on double immunofluorescence stainings, a significant colocalization between acetaldehyde and malondialdehyde adducts and histological tissue damage was observed (28). Moreover, a significant colocalization has been demonstrated between acetaldehyde adducts and the sites of collagen deposition in alcoholic liver disease (28,67).

5. PROTEIN ADDUCTS IN EXPERIMENTAL ANIMALS

5.1. Rat model

The perivenous staining pattern for acetaldehyde adducts in the liver after ethanol consumption has also been reproduced in experimental animals. Feeding of ethanol to Sprague-Dawley rats in a simultaneous pair feeding system (36% ethanol, 23% protein, 10% carbohydrate, and 32% fat) for four weeks resulted in small amounts of

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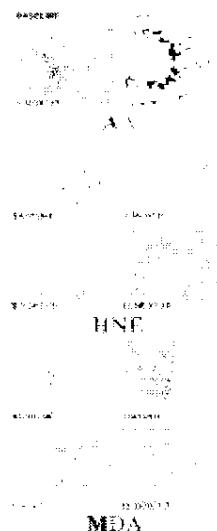
Figure 2. Sequential appearances of acetaldehyde (AA), malondialdehyde (MDA), and 4-hydroxynonenal (HNE) adducts in minipigs consuming ethanol. Biopsies were taken at 1,5, and 12 months after initiation of the ethanol diet. During follow-up abundant amounts of protein adducts were formed around the central vein coinciding with histopathological findings of steatonecrosis and focal inflammation and preceding fibrosis, which was evident at 12 months. See text for details. Immunoperoxidase staining, original magnification x 250. Reproduced with permission from Hepatology 22, 1200-1214 (76).

centrilobular and sinusoidal acetaldehyde adducts (28). Interestingly, acetaldehyde adducts were found to be most abundant in those animals, which also showed withdrawal symptoms during the course of the experiment indicating that individual high blood alcohol levels may be associated with increased amounts of adducts in tissues.

In recent experiments, where alcohol was administered to rats together with a high fat diet, distinct positive reactions for the different protein adducts were noted together with increased expression of cytochrome P450IIE1, although also with cytochrome 3A (68). Apparently, the high fat diet stimulates the formation of protein adducts with MDA and HNE. When ethanol-containing high-fat diet is further supplemented with iron a marked potentiation of adduct formation is seen together with strikingly elevated levels of serum liver-derived enzymes and progressive histopathology (69). The dietary iron supplementation to ethanol-diet in rats also results in the development of micronodular cirrhosis indicating that alcohol and iron together have a strong synergistic effect in producing liver pathology. Dietary supplementation with

iron alone in amounts producing hemochromatosis in rats has also been found to result in the appearance of lipid-peroxidation derived aldehydes, although only in small amounts (29,30). Alcohol consumption may have an additive hepatotoxic effect also in human patients with iron burden (70-74).

5.2. Micropig model

A micropig model of alcohol-induced liver disease has recently been developed by Halsted and coworkers (67,75). Such micropigs consume ethanol voluntarily while demonstrating evidence of progressive hepatic injury, including steatonecrosis and fibrosis (67,76). The sequential appearances of acetaldehyde, MDA, and HNE adducts were examined from micropig liver biopsy specimens obtained at 1, at 5, and at 12 months after the initiation of the ethanol diet (figure 2). After 1 month on the ethanol-containing diet, AA and MDA adducts were observed in zone 3 hepatocytes colocalizing with each other and appearing together with increased serum concentrations of liver-derived enzymes (76). HNE adducts were usually less intense and more diffuse and were also seen in some biopsy specimens from control animals. The most intense reactions for each adduct were seen together with evidence of steatonecrosis and focal inflammation. In terminal biopsies at 12 months, perivenous fibrosis was present in most of the biopsy specimens, which had contained perivenous adducts of AA and MDA in the early phases of follow-up, suggesting that adduct formation precedes fibrogenesis in alcohol consumers. Recently, it was further shown that the formation of protein adducts is aggravated together with the induction of several cytochrome enzymes in castrated minipigs, suggesting that sex steroid hormones also play a role in the generation of liver toxicity through such mechanisms (77).

6. PERSPECTIVE

Recent work has rendered new insights on the origin and structure of protein adducts created as a result of excessive alcohol consumption. Analysis of studies indicates that several types of chemical condensation products with proteins are generated upon heavy alcohol intake. While the formation of such adducts seem to have an important pathogenic role in creating the adverse effects of ethanol in tissues, there may also be potential diagnostic applications for more specific detection of ethanol-induced diseases. However, the rate of formation and the relative importance of the various types of adducts needs to be addressed in future studies. This approach will eventually produce fruitful results in the next few years. It also remains to be established whether prevention of adduct formation could open new possibilities for therapeutic interventions in alcoholic patients.

7. ACKNOWLEDGMENTS

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8. REFERENCES

1. Lieber CS: Metabolic effects of acetaldehyde. *Biochem Soc Trans* 16, 241-247 (1988)

2. Cederbaum AI: Role of lipid peroxidation and stress in alcohol toxicity. *Free Rad Biol Med* 7, 537-539 (1989)

3. French SW: Biochemical basis for alcohol-induced liver injury. *Clin Biochem* 22, 41-49 (1989)

4. Tsukamoto H, K. Gaal & S.W. French: Insights into the pathogenesis of alcoholic liver necrosis and fibrosis; status report. *Hepatology* 12, 599-608 (1990)

5. Nordmann R, C. Ribière & H. Rouach: Implication of free radical mechanisms in ethanol induced cellular injury. *Free Rad Biol Med* 12, 219-240 (1992)

6. Tuma DJ & M.F. Sorrell: The role of acetaldehyde adducts in liver injury. In: Alcoholic liver disease: pathology and pathogenesis. Ed: Hall P, Edward Arnold, London (1995)

7. Stevens VJ, W.J. Fantl, C.B. Newman, R.V. Sims, A. Cerami & C.M. Peterson: Acetaldehyde adducts with hemoglobin. *J Clin Invest* 67, 361-369 (1981)

8. Sorrell MF & D.J. Tuma: Hypothesis: Alcoholic liver injury and the covalent binding of acetaldehyde. *Alcohol Clin Exp Res* 9, 306-309 (1985)

9. Sorrell MF & D.J. Tuma: The functional implications of acetaldehyde binding to cell constituents. *Ann N Y Acad Sci* 492, 50-62 (1987)

10. Israel Y, E. Hurwitz, O. Niemelä & R. Arnon: Monoclonal and polyclonal antibodies against acetaldehyde-containing epitopes in acetaldehyde-protein adducts. *Proc Natl Acad Sci USA* 83, 7923-7927 (1986)

11. Lin RC, R.S. Smith & L. Lumeng: Detection of a protein-cetaldehyde adduct in the liver of rats fed alcohol chronically. *J Clin Invest* 81, 615-619 (1988)

12. Lin RC, J. Dai, L. Lumeng & M. Zhang: Serum low-density lipoprotein of alcoholic patients is chemically modified in vivo and induces apolipoprotein E synthesis by macrophages. *J Clin Invest* 95, 1979-1986 (1995)

13. Moncada C, V. Torres, G. Varghese, E. Albano & Y. Israel: Ethanol-derived immunoreactive species formed by free radical mechanisms. *Mol Pharmacol* 46, 786-791 (1994)

14. Clot P, E. Albano, E. Eliasson, M. Tabone, S. Aricò, Y. Israel, C. Moncada & M. Ingelman-Sundberg: Cytochrome P4502E1 hydroxyethyl radical adducts as the major antigen in autoantibody formation among alcoholics. *Gastroenterology* 111, 206-216 (1996)

15. Kalish GH & N.R. DiLuzio: Peroxidation of liver lipids in the pathogenesis of the ethanol-induced fatty liver. *Science* 152, 1390-1392 (1966)

16. Fraenkel-Konrad J & G. Singer: Nucleoside adducts are formed by cooperative reaction of acetaldehyde and alcohols: a possible mechanism for the role of alcohol in carcinogenesis. *Proc Natl Acad Sci USA* 85, 3959-3961 (1988)

17. Trudell JR, C.M. Ardies & W.R. Anderson: Crossreactivity of antibodies raised against acetaldehyde adducts of protein with acetaldehyde adducts of phosphatidyl-ethanolamine: Possible role in alcoholic cirrhosis. *Mol Pharmacol* 38, 587-593 (1990)

18. Donohue TM, D.J. Tuma & M.F. Sorrell: Acetaldehyde adducts with proteins: binding of [14C]acetaldehyde to serum albumin. *Arch Biochem Biophys* 220, 239-246 (1983)

19. Tuma DJ, M.R. Newman, T.M. Donohue & M.F. Sorrell: Covalent binding of acetaldehyde to proteins: participation of lysine residues. *Alcohol Clin Exp Res* 11, 579-584 (1987)

20. Jennett RB, M.F. Sorrell, A. Saffari-Fard, J.L. Ockner & D.J. Tuma: Preferential covalent binding of acetaldehyde to the α-chain of purified rat liver tubulin. *Hepatology* 9, 57-62 (1989)

21. San George RC & H.D. Hoberman: Reaction of acetaldehyde with hemoglobin. *J Biol Chem* 261, 6811-6821 (1986)

22. Fowles LF, E. Beck, S. Worrall, B.C. Shanley & J. de Jersey: The formation and stability of imidazolidinone adducts from acetaldehyde and model peptides. A kinetic study with implications for protein modification in alcohol abuse. *Biochem Pharmacol* 51, 1259-1267 (1996)

23. Palinski W, S. Ylä-Herttuala, M.E. Rosenfeld, S.W. Butler, S.A. Socher, S. Parthasarathy, L.K. Curtiss & J.L. Witztum: Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10, 325-335 (1990)

24. Esterbauer H, R. Jörgschaur & H. Zolner: Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde, and related aldehydes. *Free Rad Biol Med* 11, 81-128 (1991)

25. Stadtman ER: Protein oxidation and aging. *Science* 257, 1220-1224 (1992)

26. Haberland ME, D. Fong & L. Cheng: Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science* (Wash. DC). 241, 215-218 (1988)

27. Steinberg D, S. Parthasarathy, T.F. Carew, J.C. Khoo & J.L. Witztum: Beyond cholesterol. Modifications of low-

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density lipoprotein that increase its atherogenicity. *N Engl J Med* 320, 915-924 (1989)

28. Niemelä O, S. Parkkila, S. Ylä-Herttuala, C.H. Halsted, J.L. Witztum, A. Lanca & Y. Israel: Covalent protein adducts in the liver as a result of ethanol metabolism and lipid peroxidation. *Lab Invest* 70, 537-546 (1994)

29. Houglum K, M. Filip, J.L. Witztum & M. Chojkier: Malondialdehyde and 4-hydroxynonenal protein adducts in plasma and liver of rats with iron overload. *J Clin Invest* 86, 1991-1998 (1990)

30. Parkkila S, O. Niemelä, R.S. Britton, K.E. Brown, S. Ylä-Herttuala, R. O'Neill & B.R. Bacon: Vitamin E decreases hepatic levels of aldehyde-derived peroxidation products in rats with iron overload. *Am J Physiol* 270, G376-G384 (1996)

31. Tuma DJ, G.M. Thiele, D. Xu, L.W. Klassen & M.F. Sorrell: Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. *Hepatology* 23, 872-880 (1996)

32. Clot P, G. Bellomo, M. Tabone, S. Aricò & E. Albano: Detection of antibodies against proteins modified by hydroxyethyl free radicals in patients with alcoholic cirrhosis. *Gastroenterology* 108, 201-207 (1995)

33. Behrens UJ, M. Hoerner, J.M. Lasker & C.S. Lieber: Formation of acetaldehyde adducts with ethanol-inducible P450IIE1 in vivo. *Biochem Biophys Res Commun* 154, 584-590 (1988)

34. Castillo T, D.R. Koop, S. Kamimura, G. Triadafilopoulos & H. Tsukamoto: Role of cytochrome P4502E1 in ethanol-carbon tetrachloride- and iron-dependent microsomal lipid peroxidation. *Hepatology* 16, 992-996 (1992)

35. Nanji AA, S. Zhao, S.M.H. Sadrzadeh, A.J. Dannenberg, S.R. Tahan & D.J. Waxman: Markedly enhanced cytochrome P4502E1 induction and lipid peroxidation is associated with severe liver injury in fish oil-treated ethanol-fed rats. *Alcohol Clin Exp Res* 18, 1280-1285 (1994)

36. Zhu Y, M.J. Fillenwarth, D. Crabb, L. Lumeng & R.C. Lin: Identification of the 37-kd rat liver protein that forms an acetaldehyde adduct in vivo as delta-2-3-ketosteroid 5-beta-reductase. *Hepatology* 23, 115-122 (1996)

37. McKinnon G, J. de Jersey, B. Shanley & L. Ward: The reaction of acetaldehyde with brain microtubular proteins: Formation of stable adducts and inhibition of polymerization. *Neurosci Lett* 79, 163-168 (1987)

38. Smith SL, R.B. Jennett, M.F. Sorrell & D.J. Tuma: Acetaldehyde substoichiometrically inhibits bovine neurotubulin polymerization. *J Clin Invest* 84, 337-341 (1989)

39. Tuma DJ, S.L. Smith & M.F. Sorrell: Acetaldehyde and microtubules. *Ann N Y Acad Sci* 625, 786-792 (1991)

40. Mauch TJ, T.M. Donohue & R.K. Zetterman: Covalent binding of acetaldehyde selectively inhibits the catalytic activity of lysine-dependent enzymes. *Hepatology* 6, 263-269 (1986)

41. Mauch TJ, D.J. Tuma & M.F. Sorrell: The binding of acetaldehyde to the active site of ribonuclease: alterations in catalytic activity and effects of phosphate. *Alcohol* 22, 103-112 (1987)

42. Casey CA, S.L. Kragskow, M.F. Sorrell & D.J. Tuma: Zonal differences in ethanol-induced impairments in receptor-mediated endocytosis of asialoglycoproteins in isolated rat hepatocytes. *Hepatology* 13, 260-266 (1991)

43. Brenner DA & M. Chojkier: Acetaldehyde increases collagen gene transcription in cultured human fibroblasts. *J Biol Chem* 262, 17690-17695 (1987)

44. Chojkier M, K. Houglum, J. Solis-Herruzo & D.A. Brenner: Stimulation of collagen gene expression by ascorbic acid in cultured human fibroblast. A role for lipid peroxidation? *J Biol Chem* 264, 16957-16962 (1989)

45. Parola M, M. Pinzani, A. Casini, E. Albano, G. Poli, A. Gentilini, P. Gentilini & M.W. Dianzani: Stimulation of lipid peroxidation or 4-hydroxinonenal treatment increases procollagen a1(I) gene expression in human liver fat-storing cells. *Biochem & Biophys Res Comm* 194, 1044-1050 (1993)

46. Casini A, M. Cunningham, M. Rojkind & C.S. Lieber: Acetaldehyde increases procollagen type I and fibronectin gene transcription in cultured rat fat-storing cells through a protein synthesis-dependent mechanism. *Hepatology* 13, 758-765 (1991)

47. Casini A, E. Ceni, R. Salzano, D. Schuppan, S. Milani, G. Pellegrini & C. Surrenti: Regulation of undulin synthesis and gene expression in human fat-storing cells by acetaldehyde and transforming growth factor-beta-1: Comparison with fibronectin. *Biochem Biophys Res Commun* 199, 1019-1026 (1994)

48. Friedman DL: The cellular basis of hepatic fibrosis: mechanisms and treatment strategies. *N Engl J Med* 328, 1828-1835 (1993)

49. Lee KS, M. Buck, K. Houglum & M. Chojkier: Activation of hepatic stellate cells by TGF-alpha and collagen type I is mediated by oxidative stress through c-*myb* expression. *J Clin Invest* 96, 2461-2468 (1995)

50. Houglum K: Alcohol and iron: A radical combination. *Hepatology* 23, 1700-1703 (1996)

51. Houglum K, D.A. Brenner & M. Chojkier: d-alpha Tocopherol inhibits collagen a1(I) gene expression in cultured human fibroblasts. Modulation of constitutive collagen gene expression by lipid peroxidation. *J Clin Invest* 87, 2230-2235 (1991)

52. Bedossa P, K. Houglum, C. Trautwein, A. Holstege & M. Chojkier: Stimulation of collagen alpha-1(I) gene expression is associated with lipid peroxidation in hepatocellular injury: A link to tissue fibrosis? *Hepatology* 19, 1262-1271 (1994)

53. Niemelä O, F. Klajner, H. Orrego, E. Vidins, L. Blendis & Y. Israel: Antibodies against acetaldehyde-modified protein epitopes in human alcoholics. *Hepatology* 7, 1210-1214 (1987)

54. Hoerner M, U.J. Behrens, T.M. Worner, I. Blacksberg, R. Braley, F. Schaffner & C.S. Lieber: The role of alcoholism and liver disease in the appearance of serum antibodies against acetaldehyde adducts. *Hepatology* 8, 569-574 (1988)

55. Izumi N, Y. Sakai, W. Koyama & Y. Hasumura: Clinical significance of serum antibodies against alcohol-altered hepatocyte membrane in alcoholic liver disease. *Alcohol Clin Exp Res* 13, 762-765 (1989)

56. Worrall S, J. de Jersey, B.C. Shanley & P.A. Wilce: Antibodies against acetaldehyde-modified epitopes: an elevated IgA response in alcoholics. *Eur J Clin Invest* 21, 90-95 (1991)

57. Koskinas J, J.G. Kenna, G.L. Bird, G.J.M. Alexander & R. Williams: Immunoglobulin A antibody to a 200-kilodalton cytosolic acetaldehyde adduct in alcoholic hepatitis. *Gastroenterology* 103, 1860-1867 (1992)

58. Wehr A, M. Rodo, C.S. Lieber & E. Baraona: Acetaldehyde adducts and autoantibodies against VLDL and LDL in alcoholics. *J Lipid Res* 34, 1237-1244 (1993)

59. Xu D, G.M. Thiele, J.L. Beckenhauer, L.W. Klassen, M.F. Sorrell & D.J. Tuma: Detection of circulating antibodies to malondialdehyde-acetaldehyde adducts in ethanol-fed rats. *Gastroenterology* 115, 686-692 (1998)

60. Viitala K, J.E. Blake, Y. Israel & O. Niemelä: Serum IgA, IgG, and IgM antibodies directed against acetaldehyde-derived epitopes: Relationship to liver disease severity and alcohol consumption. *Hepatology* 25, 1418-1424 (1997)

61. Yokoyama H, S. Nagata, S. Moriya, S. Kato, K. Ito, K. Kamegaya & H. Ishii: Hepatic fibrosis produced in guinea pigs by chronic ethanol administration and immunization with acetaldehyde adducts. *Hepatology* 21, 1438-1442 (1995)

62. Niemelä O & Y. Israel: Hemoglobin-acetaldehyde adducts in human alcohol abusers. *Lab Invest* 67, 246-252 (1992)

63. Lin RC, S. Shahidi, T.J. Kelly, C. Lumeng & L. Lumeng: Measurement of hemoglobin-acetaldehydeadduct in alcoholic patients. *Alcohol Clin Exp Res* 17, 669-674 (1993)

64. Niemelä O: Acetaldehyde adducts of proteins: Diagnostic and pathogenic implications in diseases caused by excessive alcohol consumption. *Scand J Clin Lab Invest* 53, 45-54 (1993)

65. Niemelä O, T. Juvonen & S. Parkkila: Immunohistochemical demonstration of acetaldehyde containing epitopes in human liver after ethanol ingestion. *J Clin Invest* 87, 1367-1374 (1991)

66. Holstege A, P. Bedossa, T. Poynard, M. Kollinger, J.C. Chaput, K. Houglum & M. Chojkier: Acetaldehyde-modified epitopes in liver biopsy specimens of alcoholic and nonalcoholic patients: localization and association with progression of liver fibrosis. *Hepatology* 19, 367-372 (1994)

67. Halsted CH, J. Villanueva, C.J. Chandler, B. Ruebner, R.J. Munn, S. Parkkila & O. Niemelä: Centrilobular distribution of acetaldehyde and collagen in the ethanol-fed micropig. *Hepatology* 18, 954-960 (1993)

68. Niemelä O, S. Parkkila, M. Pasanen, Y. Iimuro, B. Bradford & R.G. Thurman: Early alcoholic liver injury: Formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. *Alcohol Clin Exp Res* 22, 2118-2124 (1998)

69. Tsukamoto H, W. Horne, S. Kamimura, O. Niemelä, S. Parkkila, S. Ylä-Herttuala & G.M. Brittenham: Experimental liver disease induced by alcohol and iron. *J Clin Invest* 96, 620-630 (1995)

70. Bassett ML, J.W. Halliday & L.W. Powell: Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology* 6, 24-29 (1986)

71. Irving MG J.W. Halliday & L.W. Powell: Association between alcoholism and increased hepatic iron stores. *Alcohol Clin Exp Res* 12, 7-13 (1988)

72. Kukielka E & A.I. Cederbaum: NADPH- and NADH-dependent oxygen radical generation by rat liver nuclei in the presence of redox cycling agents and iron. *Arch Biochem Biophys* 283, 326-333 (1990)

73. Adams PC & S. Agnew: Alcoholism in hereditary hemochromatosis revisited: Prevalence and clinical consequences among homozygous siblings. *Hepatology* 23, 724-727 (1996)

74. Niemelä O, S. Parkkila, R.S. Britton, E. Brunt, C. Janney & B.R. Bacon: Hepatic lipid peroxidation in hereditary hemochromatosis. *J Lab Clin Med*, in press (1999)

75. Zidenberg-Cherr S, K.L. Olin, J. Villanueva, A. Tang, S. Phinney, C.H. Halsted & C.L. Keen: Ethanol-induced changes in hepatic free radical defence mechanisms and fatty acid composition in the miniature pig. *Hepatology* 13, 1185-1192 (1991)

76. Niemelä O, S. Parkkila, S. Ylä-Herttuala, J. Villanueva, B. Ruebner & C.H. Halsted: Sequential acetaldehyde production, lipid peroxidation and fibrogenesis in micropig model of alcohol-induced liver disease. *Hepatology* 22, 1208-1214 (1995)

77. Niemelä O, S. Parkkila, M. Pasanen, K. Viitala, J.A. Villanueva & C.H. Halsted: Induction of CYP enzymes and generation of protein-aldehyde adducts are associated with sex steroid dependent sensitivity to alcoholic liver disease in micropigs. Manuscript, submitted (1999)

78. Nomura F & C.S. Lieber: Binding of acetaldehyde to rat liver microsomes: enhancement after chronic alcohol consumption. *Biochem Biophys Res Commun* 100, 131-137 (1981)

79. Medina VA, T. M. Donohue, M.F. Sorrell & D.J. Tuma: Covalent binding of acetaldehyde to hepatic proteins during ethanol oxidation. *J Lab Clin Med* 105, 5-10 (1985)

80. French SW, K. Wong, L. Jui, E. Albano, A.-L. Hagbjörk & M. Ingelman-Sundberg: Effect of ethanol on cytochrome P450 (CYP2E1), lipid peroxidation and serum protein adduct formation in relation to liver pathology pathogenesis. *Exp Mol Pathol* 58, 61-75 (1993)

81. Kamimura S, K. Gaal, R.S. Britton, B.R. Bacon, G. Triadafilopoulos & H. Tsukamoto: Increased 4-hydroxynonenal levels in experimental alcoholic liver disease: Association of lipid peroxidation with liver fibrogenesis. *Hepatology* 16, 448-453 (1992)

82. Chen J, S. Schenker, T.A. Frosto & G.I. Henderson: Inhibition of cytochrome c oxidase activity by 4-hydroxynonenal (HNE). Role of HNE adduct formation with the enzyme subunits. *Biochim Biophys Acta* 1380, 336-44 (1998)

83. Ohhira M, T. Ohtake, A. Matsumoto, H. Saito, K. Ikuta, Y. Fujimoto, M. Ono, S. Toyokuni & Y. Kohgo: Immunohistochemical detection of 4-hydroxy-2-nonenal-modified-protein adducts in human alcoholic liver diseases. *Alcohol Clin Exp Res* 22, 1455-1495 (1998)

84. Clot P, M. Parola, G. Bellomo, U. Dianzani, R. Carini, M. Tabone, S. Arico, M. Ingelman-Sundberg & E. Albano: Plasma membrane hydroxyethyl radical adducts cause antibody-dependent cytotoxicity in rathepatocytes exposed to alcohol. *Gastroenterology* 113, 265-276 (1997)

85. Albano E, P. Clot, M. Morimoto, A. Tomasi, M. Ingelman-Sundberg & S.W. French: Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol. *Hepatology* 23, 155-163 (1996)

86. Gaines K, J. Salhany, D. Tuma & M.F. Sorrell: Reactions of acetaldehyde with human erythrocyte membrane proteins. *Febs Lett* 75, 115-119 (1977)

87. Peterson CM & B.K. Scott: Studies of whole blood associated acetaldehyde as a marker for alcohol intake in mice. *Alcohol Clin Exp Res* 13, 845-847 (1989)

88. Jukkola A & O. Niemelä: Covalent binding of acetaldehyde to type III collagen. *Biochem Biophys Res Commun* 159, 163-169 (1989)

89. Behrens UJ, X-L. Ma, E. Baraona & C.S. Lieber: Acetaldehyde-collagen adducts in CC14-induced liver injury in rats. *Hepatology* 10, 608a (1989)

90. Paradis V, P. Mathurin, V. Ratziu, T. Poynard & P. Bedossa: Binding of apolipoprotein A-I and acetaldehyde-modified apolipoprotein A-I to liver extracellular matrix. *Hepatology* 23, 1232-1238 (1996)

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